



**xx**  
**cc** The invention relates to a composition comprising several cDNAs that are  
**cc** differentially expressed in a liver disorder. The composition is useful  
**cc** for treating liver disorder such as hyperlipidemia, hypertension, type  
**cc** II diabetes, tumours of the liver and disorders of the inflammatory and  
**cc** immune response. The composition is useful for a high-throughput method  
**cc** of screening several molecule or compounds to identify a ligand which  
**cc** specifically binds a cDNA. A protein encoded by the cDNA is useful for a  
**cc** high-throughput method for using a protein to screen several molecules or  
**cc** compounds to identify at least one ligand which specifically binds the  
**cc** protein which involves combining the protein encoded by the cDNA with  
**cc** several of molecules or compounds under condition to allow specific  
**cc** binding and detecting specific binding between the protein and a  
**cc** molecule or compound, therefor identifying a ligand which specifically  
**cc** binds the protein. The composition is useful for detecting and  
**cc** quantifying differential gene expression, can be used in gene therapy, to  
**cc** formulate prognosis and to design a treatment regimen and to monitor the  
**cc** efficacy of treatment. The present sequence represents a cDNA  
**cc** differentially expressed in a liver disorder.

SQ

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**Qy** 1 ATGGAGCCGGCAGGACCCAGATACTTGACCCAGGTACAGGAGTCTTGAG 60  
**Db** 37 ATGGAGCCGGCAGGACCCAGATACTTGACCCAGGTACAGGAGTCTTGAG 96  
**Qy** 61 GTGCGAGAACCAATTACGCATCCCTCGCCCTCTCTCAACCTCCACAGGCC 120  
**Db** 97 GTGCTGAGAACCAATTACGCATCCCTCGCCCTCTCAACCTCCACAGGCC 156  
**Qy** 121 CAACTCTGAGGCCTGACCTCTGACTTCCCTCACTAGCATCTGACCTTGCG 180  
**Db** 157 C-ACTCTGAGGCCTGACCTCTGACTTCCCTCACTAGCATCTGACCTTGCG 215  
**Qy** 181 GCGCTGGCCATTCATCTGCATCTGAGGATGCGCTACTGTAAGAACCCRT 240  
**Db** 216 GCGCTGGCCATTCATCTGCATCTGAGGATGCGCTACTGTAAGAACCCRT 275  
**Qy** 241 TGCCCATGAGGGAGGACTCTGCTCTGGAGAGCTCGACCCAGGGGTGCTG 300  
**Db** 276 TGCCCATGAGGGAGGACTCTGCTCTGGAGAGCTCGACCCAGGGGTGCTG 335  
**Qy** 301 CTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 360  
**Db** 336 CTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 395  
**Qy** 361 TGCTTTATGCTGCTGCTTACCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 420  
**Db** 396 TGCTTTATGCTGCTGCTTACCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 455  
**Qy** 421 AGGAGSCAGGTGAGGACCTGAGGACCCCCCTTGAGATAGCTGAGGCTGCTG 480  
**Db** 456 AGGAGSCAGGTGAGGACCTGAGGACCCCCCTTGAGATAGCTGAGGCTGCTG 515  
**Qy** 481 TCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 540  
**Db** 516 TCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTG 575  
**Qy** 541 ATGTTGACCCCTTCATACCGCTTGTGAGATAGCTGAGGCTGCTGCTGCTG 600  
**Db** 575 ATGTTGACCCCTTCATACCGCTTGTGAGATAGCTGCTGCTGCTGCTGCTGCTG 634  
**Qy** 601 CTGCTCCGAGGACATC-TATGACCCAGACATTCTGAGGGGGCACGCTATG 659  
**Db** 635 CTGCTCCGAGGACATC-TATGACCCAGACATTCTGAGGGGGCACGCTATG 694  
**Qy** 660 GATCAACACTTCCTGGCTCCACCTCTGCTCTGGACCTGGACATCATTC 719  
**Db** 695 GATCAACACTTCCTGGCTCCACCTCTGCTCTGGACCTGGACATCATTC 754

**Db** 576 ATGTTGACCCCTTCATACCGCTTGTGAGATAGCTGCTGCTGCTGCTGCTG 634  
**Qy** 601 CTGCTCCGAGGACATC-TATGACCCAGACATTCTGAGGGGGCACGCTATG 659  
**Db** 635 CTGCTCCGAGGACATC-TATGACCCAGACATTCTGAGGGGGCACGCTATG 694  
**Qy** 660 GATCAACACTTCCTGGCTCCACCTCTGCTCTGGACCTGGACATCATTC 719  
**Db** 695 GATCAACACTTCCTGGCTCCACCTCTGCTCTGGACCTGGACATCATTC 754

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**xx**  
**pt** Composition comprising several cDNAs that are differentially expressed in  
**pt** treated human C3A liver cell cultures, useful for treating liver  
**pt** disorder.

PS : Claim 1; SEQ ID NO 94; 41PP; English.



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(54) Title: HUMAN TRANSMEMBRANE PROTEINS</p> <p>(57) Abstract</p> <p>The invention provides human transmembrane proteins (HTMPN) and polynucleotides which identify and encode HTMPN. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of HTMPN.</p>																															

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## HUMAN TRANSMEMBRANE PROTEINS

### TECHNICAL FIELD

5 This invention relates to nucleic acid and amino acid sequences of human transmembrane proteins and to the use of these sequences in the diagnosis, treatment, and prevention of immune, reproductive, smooth muscle, neurological, gastrointestinal, developmental, and cell proliferative disorders.

### 10 BACKGROUND OF THE INVENTION

Eukaryotic organisms are distinct from prokaryotes in possessing many intracellular organelle and vesicle structures. Many of the metabolic reactions which distinguish eukaryotic biochemistry from prokaryotic biochemistry take place within these structures. In particular, many cellular functions require very stringent reaction conditions, and the organelles and vesicles enable compartmentalization and isolation of reactions which might otherwise disrupt cytosolic metabolic processes. The organelles include mitochondria, smooth and rough endoplasmic reticula, sarcoplasmic reticulum, and the Golgi body. The vesicles include phagosomes, lysosomes, endosomes, peroxisomes, and secretory vesicles. Organelles and vesicles are bounded by single or double membranes.

Biological membranes are highly selective permeable barriers made up of lipid bilayer sheets composed of phosphoglycerides, fatty acids, cholesterol, phospholipids, glycolipids, proteoglycans, and proteins. Membranes contain ion pumps, ion channels, and specific receptors for external stimuli which transmit biochemical signals across the membranes. These membranes also contain second messenger proteins which interact with these pumps, channels, and receptors to amplify and regulate transmission of these signals.

#### Plasma Membrane Proteins

Plasma membrane proteins (MPs) are divided into two groups based upon methods 30 of protein extraction from the membrane. Extrinsic or peripheral membrane proteins can be released using extremes of ionic strength or pH, urea, or other disruptors of protein interactions. Intrinsic or integral membrane proteins are released only when the lipid

bilayer of the membrane is dissolved by detergent.

- Transmembrane proteins (TM) are characterized by an extracellular, a transmembrane, and an intracellular domain. TM domains are typically comprised of 15 to 25 hydrophobic amino acids which are predicted to adopt an  $\alpha$ -helical conformation.
- 5 TM proteins are classified as bitopic (Types I and II) proteins, which span the membrane once, and polytopic (Types III and IV) (Singer, S.J. (1990) Annu. Rev. Cell Biol. 6:247-96) proteins which contain multiple membrane-spanning segments. TM proteins that act as cell-surface receptor proteins involved in signal transduction include growth and differentiation factor receptors, and receptor-interacting proteins such as *Drosophila*
- 10 pecanex and frizzled proteins, LIV-1 protein, NF2 protein, and GNS1/SUR4 eukaryotic integral membrane proteins. TM proteins also act as transporters of ions or metabolites, such as gap junction channels (connexins), and ion channels, and as cell anchoring proteins, such as lectins, integrins, and fibronectins. TM proteins are found in vesicle organelle-forming molecules, such as calveolins; or cell recognition molecules, such as
- 15 cluster of differentiation (CD) antigens, glycoproteins, and mucins.

Many membrane proteins (MPs) contain amino acid sequence motifs that serve to localize proteins to specific subcellular sites. Examples of these motifs include PDZ domains, KDEL, RGD, NGR, and GSL sequence motifs, von Willebrand factor A (vWFA) domains, and EGF-like domains. RGD, NGR, and GSL motif-containing peptides have been used as drug delivery agents in targeted cancer treatment of tumor vasculature (Arap, W. et al. (1998) Science, 279:377-380). Membrane proteins may also contain amino acid sequence motifs that serve to interact with extracellular or intracellular molecules, such as carbohydrate recognition domains.

Chemical modification of amino acid residue side chains alters the manner in which MPs interact with other molecules, for example, phospholipid membranes. Examples of such chemical modifications to amino acid residue side chains are covalent bond formation with glycosaminoglycans, oligosaccharides, phospholipids, acetyl and palmitoyl moieties, ADP-ribose, phosphate, and sulphate groups.

RNA-encoding membrane proteins may have alternative splice sites which give rise to proteins encoded by the same gene but with different messenger RNA and amino acid sequences. Splice variant membrane proteins may interact with other ligand and protein isoforms.

G-Protein Coupled Receptors

G-protein coupled receptors (GPCR) are a superfamily of integral membrane proteins which transduce extracellular signals. GPCRs include receptors for biogenic amines, lipid mediators of inflammation, peptide hormones, and sensory signal mediators.

- 5       The structure of these highly-conserved receptors consists of seven hydrophobic transmembrane (serpentine) regions, cysteine disulfide bridges between the second and third extracellular loops, an extracellular N-terminus, and a cytoplasmic C-terminus. Three extracellular loops alternate with three intracellular loops to link the seven transmembrane regions. The most conserved parts of these proteins are the  
10      transmembrane regions and the first two cytoplasmic loops. A conserved, acidic-Arg-aromatic residue triplet present in the second cytoplasmic loop may interact with G proteins. A GPCR consensus pattern is characteristic of most proteins belonging to this superfamily (ExPASy PROSITE document PS00237; and Watson, S. and S. Arkinstall (1994) The G-protein Linked Receptor Facts Book, Academic Press, San Diego,  
15      CA, pp 2-6). Mutations and changes in transcriptional activation of GPCR-encoding genes have been associated with neurological disorders such as schizophrenia, Parkinson's disease, Alzheimer's disease, drug addiction, and feeding disorders.

Scavenger Receptors

- Macrophage scavenger receptors with broad ligand specificity may participate in  
20      the binding of low density lipoproteins (LDL) and foreign antigens. Scavenger receptors types I and II are trimeric membrane proteins with each subunit containing a small N-terminal intracellular domain, a transmembrane domain, a large extracellular domain, and a C-terminal cysteine-rich domain. The extracellular domain contains a short spacer domain, an  $\alpha$ -helical coiled-coil domain, and a triple helical collagenous domain. These  
25      receptors have been shown to bind a spectrum of ligands, including chemically modified lipoproteins and albumin, polyribonucleotides, polysaccharides, phospholipids, and asbestos (Matsumoto, A. et al. (1990) Proc. Natl. Acad. Sci. 87:9133-9137; and Elomaa, O. et al. (1995) Cell 80:603-609). The scavenger receptors are thought to play a key role in atherogenesis by mediating uptake of modified LDL in arterial walls, and in host  
30      defense by binding bacterial endotoxins, bacteria, and protozoa.

Tetraspan family proteins

The transmembrane 4 superfamily (TM4SF) or tetraspan family is a multigene

family encoding type III integral membrane proteins (Wright, M.D. and Tomlinson, M.G. (1994) *Immunol. Today* 15:588). TM4SF is comprised of membrane proteins which traverse the cell membrane four times. Members of the TM4SF include platelet and endothelial cell membrane proteins, melanoma-associated antigens, leukocyte surface 5 glycoproteins, colonial carcinoma antigens, tumor-associated antigens, and surface proteins of the schistosome parasites (Jankowski, S.A. (1994) *Oncogene* 9:1205-1211). Members of the TM4SF share about 25-30% amino acid sequence identity with one another.

A number of TM4SF members have been implicated in signal transduction, control of cell adhesion, regulation of cell growth and proliferation, including development and 10 oncogenesis, and cell motility, including tumor cell metastasis. Expression of TM4SF proteins is associated with a variety of tumors and the level of expression may be altered when cells are growing or activated.

#### Tumor Antigens

Tumor antigens are surface molecules that are differentially expressed in tumor 15 cells relative to normal cells. Tumor antigens distinguish tumor cells immunologically from normal cells and provide diagnostic and therapeutic targets for human cancers (Takagi, S. et al. (1995) *Int. J. Cancer* 61: 706-715; Liu, E. et al. (1992) *Oncogene* 7: 1027-1032).

#### Ion channels

20 Ion channels are found in the plasma membranes of virtually every cell in the body. For example, chloride channels mediate a variety of cellular functions including regulation of membrane potentials and absorption and secretion of ions across epithelial membranes. When present in intracellular membranes of the Golgi apparatus and endocytic vesicles, chloride channels also regulate organelle pH (see, e.g., Greger, R. 25 (1988) *Annu. Rev. Physiol.* 50:111-122). Electrophysiological and pharmacological properties of chloride channels, including ion conductance, current-voltage relationships, and sensitivity to modulators, suggest that different chloride channels exist in muscles, neurons, fibroblasts, epithelial cells, and lymphocytes.

Many channels have sites for phosphorylation by one or more protein kinases 30 including protein kinase A, protein kinase C, tyrosine kinase, and casein kinase II, all of which regulate ion channel activity in cells. Inappropriate phosphorylation of proteins in cells has been linked to changes in cell cycle progression and cell differentiation. Changes

in the cell cycle have been linked to induction of apoptosis or cancer. Changes in cell differentiation have been linked to diseases and disorders of the reproductive system, immune system, and skeletal muscle.

**Proton pumps**

- 5 Proton ATPases are a large class of membrane proteins that use the energy of ATP hydrolysis to generate an electrochemical proton gradient across a membrane. The resultant gradient may be used to transport other ions across the membrane ( $\text{Na}^+$ ,  $\text{K}^+$ , or  $\text{Cl}^-$ ) or to maintain organelle pH. Proton ATPases are further subdivided into the mitochondrial F-ATPases, the plasma membrane ATPases, and the vacuolar ATPases.
- 10 The vacuolar ATPases establish and maintain an acidic pH within various vesicles involved in the processes of endocytosis and exocytosis (Mellman, I. et al. (1986) *Ann. Rev. Biochem.* 55:663-700).

Proton-coupled, 12 membrane-spanning domain transporters such as PEPT 1 and PEPT 2 are responsible for gastrointestinal absorption and for renal reabsorption of peptides using an electrochemical  $\text{H}^+$  gradient as the driving force. Another type of peptide transporter, the TAP transporter, is a heterodimer consisting of TAP 1 and TAP 2 and is associated with antigen processing. Peptide antigens are transported across the membrane of the endoplasmic reticulum by TAP so they can be expressed on the cell surface in association with MHC molecules. Each TAP protein consists of multiple hydrophobic membrane spanning segments and a highly conserved ATP-binding cassette (Boll, M. et al. (1996) *Proc. Natl. Acad. Sci.* 93:284-289). Pathogenic microorganisms, such as herpes simplex virus, may encode inhibitors of TAP-mediated peptide transport in order to evade immune surveillance (Marusina, K. and Manaco, J.J. (1996) *Curr. Opin. Hematol.* 3:19-26).

25 **ABC Transporters**

The ATP-binding cassette (ABC) transporters, also called the "traffic ATPases", comprise a superfamily of membrane proteins that mediate transport and channel functions in prokaryotes and eukaryotes (Higgins, C.F. (1992) *Annu. Rev. Cell Biol.* 8:67-113). ABC proteins share a similar overall structure and significant sequence homology. All ABC proteins contain a conserved domain of approximately two hundred amino acid residues which includes one or more nucleotide binding domains. Mutations in ABC transporter genes are associated with various disorders, such as hyperbilirubinemia

IL/Dubin-Johnson syndrome, recessive Stargardt's disease, X-linked adrenoleukodystrophy, multidrug resistance, celiac disease, and cystic fibrosis.

Membrane Proteins Associated with Intercellular Communication

Intercellular communication is essential for the development and survival of multicellular organisms. Cells communicate with one another through the secretion and uptake of protein signaling molecules. The uptake of proteins into the cell is achieved by endocytosis, in which the interaction of signaling molecules with the plasma membrane surface, often via binding to specific receptors, results in the formation of plasma membrane-derived vesicles that enclose and transport the molecules into the cytosol. The secretion of proteins from the cell is achieved by exocytosis, in which molecules inside of the cell are packaged into membrane-bound transport vesicles derived from the *trans*-Golgi network. These vesicles fuse with the plasma membrane and release their contents into the surrounding extracellular space. Endocytosis and exocytosis result in the removal and addition of plasma membrane components and the recycling of these components is essential to maintain the integrity, identity, and functionality of both the plasma membrane and internal membrane-bound compartments.

Lysosomes are the site of degradation of intracellular material during autophagy and of extracellular molecules following endocytosis. Lysosomal enzymes are packaged into vesicles which bud from the *trans*-Golgi network. These vesicles fuse with endosomes to form the mature lysosome in which hydrolytic digestion of endocytosed material occurs. Lysosomes can fuse with autophagosomes to form a unique compartment in which the degradation of organelles and other intracellular components occurs. Protein sorting by transport vesicles, such as the endosome, has important consequences for a variety of physiological processes including cell surface growth, the biogenesis of distinct intracellular organelles, endocytosis, and the controlled secretion of hormones and neurotransmitters (Rothman, J.E. and Wieland, F.T. (1996) *Science* 272:227-234). In particular, neurodegenerative disorders and other neuronal pathologies are associated with biochemical flaws during endosomal protein sorting or endosomal biogenesis (Mayer R.J. et al. (1996) *Adv. Exp. Med. Biol.* 389:261-269).

Peroxisomes are organelles independent from the secretory pathway. They are the site of many peroxide-generating oxidative reactions in the cell. Peroxisomes are unique among eukaryotic organelles in that their size, number, and enzyme content vary

depending upon organism, cell type, and metabolic needs. The majority of peroxisome-associated proteins are membrane-bound or are found proximal to the cytosolic or the luminal side of the peroxisome membrane (Waterham, H.R. and Cregg, J.M. (1997) BioEssays 19:57-66).

5        Genetic defects in peroxisome proteins which result in peroxisomal deficiencies have been linked to a number of human pathologies, including Zellweger syndrome, rhizomelic chondroplasia punctata, X-linked adrenoleukodystrophy, acyl-CoA oxidase deficiency, bifunctional enzyme deficiency, classical Refsum's disease, DHAP alkyl transferase deficiency, and acatalasemia (Moser, H.W. and Moser, A.B. (1996) Ann. NY Acad. Sci. 804:427-441). In addition, Gartner, J. et al. (1991; Pediatr. Res. 29:141-146) found a 22 kDa integral membrane protein associated with lower density peroxisome-like subcellular fractions in patients with Zellweger syndrome.

Normal embryonic development and control of germ cell maturation is modulated by a number of secretory proteins which interact with their respective membrane-bound receptors. Cell fate during embryonic development is determined by members of the activin/TGF- $\beta$  superfamily, cadherins, IGF-2, and other morphogens. In addition, proliferation, maturation, and redifferentiation of germ cell and reproductive tissues are regulated, for example, by IGF-2, inhibins, activins, and follistatins (Petruglia, F. (1997) Placenta 18:3-8; Mather, J.P. et al. (1997) Proc. Soc. Exp. Biol. Med. 215:209-222).

## 20 Endoplasmic Reticulum Membrane Proteins

The normal functioning of the eukaryotic cell requires that all newly synthesized proteins be correctly folded, modified, and delivered to specific intra- and extracellular sites. Newly synthesized membrane and secretory proteins enter a cellular sorting and distribution network during or immediately after synthesis and are routed to specific locations inside and outside of the cell. The initial compartment in this process is the endoplasmic reticulum (ER) where proteins undergo modifications such as glycosylation, disulfide bond formation, and assembly into oligomers. The modified proteins are then transported through a series of membrane-bound compartments which include the various cisternae of the Golgi complex, where further carbohydrate modifications occur.

30 Transport between compartments occurs by means of vesicles that bud and fuse in a manner specific to the type of protein being transported. Once within the secretory pathway, proteins do not have to cross a membrane to reach the cell surface.

Although the majority of proteins processed through the ER are transported out of the organelle, some are retained. The signal for retention in the ER in mammalian cells consists of the tetrapeptide sequence, KDEL, located at the carboxyl terminus of proteins (Munro, S. (1986) Cell 46:291-300). Proteins containing this sequence leave the ER but 5 are quickly retrieved from the early Golgi cisternae and returned to the ER, while proteins lacking this signal continue through the secretory pathway.

Disruptions in the cellular secretory pathway have been implicated in several human diseases. In familial hypercholesterolemia the low density lipoprotein receptors remain in the ER, rather than moving to the cell surface (Pathak, R.K. (1988) J. Cell Biol. 10 106:1831-1841). Altered transport and processing of the  $\beta$ -amyloid precursor protein ( $\beta$ APP) involves the putative vesicle transport protein presenilin, and may play a role in early-onset Alzheimer's disease (Levy-Lahad, E. et al. (1995) Science 269:973-977). Changes in ER-derived calcium homeostasis have been associated with diseases such as cardiomyopathy, cardiac hypertrophy, myotonic dystrophy, Brody disease, Smith-McCort dysplasia, and diabetes mellitus.

#### Mitochondrial Membrane Proteins

The mitochondrial electron transport (or respiratory) chain is a series of three enzyme complexes in the mitochondrial membrane that is responsible for the transport of electrons from NADH to oxygen and the coupling of this oxidation to the synthesis of 20 ATP (oxidative phosphorylation). ATP then provides the primary source of energy for driving the many energy-requiring reactions of a cell.

Most of the protein components of the mitochondrial respiratory chain are the products of nuclear encoded genes that are imported into the mitochondria and the remainder are products of mitochondrial genes. Defects and altered expression of 25 enzymes in the respiratory chain are associated with a variety of disease conditions in man, including, for example, neurodegenerative diseases, myopathies, and cancer.

#### Lymphocyte and Leukocyte Membrane Proteins

The B-cell response to antigens, which is modulated through receptors, is an essential component of the normal immune system. Mature B cells recognize foreign 30 antigens through B cell receptors (BCR) which are membrane-bound, specific antibodies that bind foreign antigens. The antigen/receptor complex is internalized and the antigen is proteolytically processed. To generate an efficient response to complex antigens, the

BCR, BCR-associated proteins, and T cell response are all required. Proteolytic fragments of the antigen are complexed with major histocompatibility complex-II (MHCII) molecules on the surface of the B cells where the complex can be recognized by T cells. In contrast, macrophages and other lymphoid cells present antigens in association with 5 MHC I molecules to T cells. T cells recognize and are activated by the MHC I-antigen complex through interactions with the T cell receptor/CD3 complex, a T cell-surface multimeric protein located in the plasma membrane. T cells activated by antigen presentation secrete a variety of lymphokines that induce B cell maturation and T cell proliferation and activate macrophages, which kill target cells.

10 Leukocytes have a fundamental role in the inflammatory and immune response and include monocytes/macrophages, mast cells, polymorphonucleoleukocytes, natural killer cells, neutrophils, eosinophils, basophils, and myeloid precursors. Leukocyte membrane proteins include members of the CD antigens, N-CAM, I-CAM, human leukocyte antigen (HLA) class I and HLA class II gene products, immunoglobulins, immunoglobulin 15 receptors, complement, complement receptors, interferons, interferon receptors, interleukin receptors, and chemokine receptors.

Abnormal lymphocyte and leukocyte activity has been associated with acute disorders, such as AIDS, immune hypersensitivity, leukemias, leukopenia, systemic lupus, granulomatous disease, and eosinophilia.

#### 20 Apoptosis-Associated Membrane Proteins

A variety of ligands, receptors, enzymes, tumor suppressors, viral gene products, pharmacological agents, and inorganic ions have important positive or negative roles in regulating and implementing the apoptotic destruction of a cell. Although some specific components of the apoptotic pathway have been identified and characterized, many 25 interactions between the proteins involved are undefined, leaving major aspects of the pathway unknown.

A requirement for calcium in apoptosis was previously suggested by studies showing the involvement of calcium levels in DNA cleavage and Fas-mediated cell death (Hewish, D.R. and L.A. Burgoine (1973) Biochem. Biophys. Res. Comm. 52:504-510; 30 Vignaux, F. et al. (1995) J. Exp. Med. 181:781-786; Oshimi, Y. and S. Miyazaki (1995) J. Immunol. 154:599-609). Other studies show that intracellular calcium concentrations increase when apoptosis is triggered in thymocytes by either T cell receptor cross-linking

or by glucocorticoids and cell death can be prevented by blocking this increase (McConkey, D.J. et al. (1989) J. Immunol. 143:1801-1806; McConkey, D.J. et al. (1989) Arch. Biochem. Biophys. 269:365-370). Therefore, membrane proteins such as calcium channels are important for the apoptotic response.

##### 5. Tumorigenesis

Tumorigenesis is associated with the activation of oncogenes which are derived from normal cellular genes. These oncogenes encode oncoproteins which are capable of converting normal cells into malignant cells. Some oncoproteins are mutant isoforms of the normal protein and other oncoproteins are abnormally expressed with respect to 10 location or level of expression. The latter category of oncoprotein causes cancer by altering transcriptional control of cell proliferation. Five classes of oncoproteins are known to affect the cell cycle controls. These classes include growth factors, growth factor receptors, intracellular signal transducers, nuclear transcription factors, and cell-cycle control proteins. These proteins include those which are modified by glycosylation, 15 phosphorylation, glycosaminoglycan attachment, sulphation, and lipidation.

Modulation of factors which act in the coordination of the human cell division cycle may provide an important means to reduce tumorigenesis. An example of the metastasis-associated proteins is the lysosomal membrane glycoprotein P2B/LAMP-1 which is also expressed in normal tissues. (Heffernan, M. et al. (1989) Cancer Res. 49:6077-6084.) In addition, mammalian proteins homologous to the plant pathogenesis-related proteins have been identified in hyperplastic glioma. (Murphy, E.V. et al. (1995) Gene 159:131-135.)

The discovery of new human transmembrane proteins and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful 20 in the diagnosis, prevention, and treatment of immune, reproductive, smooth muscle, 25 neurological, gastrointestinal, developmental, and cell proliferative disorders.

#### SUMMARY OF THE INVENTION

30 The invention features substantially purified polypeptides, human transmembrane proteins, referred to collectively as "HTMPN" and individually as "HTMPN-1", "HTMPN-2", "HTMPN-3", "HTMPN-4", "HTMPN-5", "HTMPN-6", "HTMPN-7", "HTMPN-8", "HTMPN-9", "HTMPN-10", "HTMPN-11", "HTMPN-12", "HTMPN-13",

"HTMPN-14", "HTMPN-15", "HTMPN-16", "HTMPN-17", "HTMPN-18", "HTMPN-19", "HTMPN-20", "HTMPN-21", "HTMPN-22", "HTMPN-23", "HTMPN-24",  
"HTMPN-25", "HTMPN-26", "HTMPN-27", "HTMPN-28", "HTMPN-29", "HTMPN-30", "HTMPN-31", "HTMPN-32", "HTMPN-33", "HTMPN-34", "HTMPN-35",  
5 "HTMPN-36", "HTMPN-37", "HTMPN-38", "HTMPN-39", "HTMPN-40", "HTMPN-41", "HTMPN-42", "HTMPN-43", "HTMPN-44", "HTMPN-45", "HTMPN-46",  
"HTMPN-47", "HTMPN-48", "HTMPN-49", "HTMPN-50", "HTMPN-51", "HTMPN-52", "HTMPN-53", "HTMPN-54", "HTMPN-55", "HTMPN-56", "HTMPN-57",  
"HTMPN-58", "HTMPN-59", "HTMPN-60", "HTMPN-61", "HTMPN-62", "HTMPN-63", "HTMPN-64", "HTMPN-65", "HTMPN-66", "HTMPN-67", "HTMPN-68",  
"HTMPN-69", "HTMPN-70", "HTMPN-71", "HTMPN-72", "HTMPN-73", "HTMPN-74", "HTMPN-75", "HTMPN-76", "HTMPN-77", "HTMPN-78", and "HTMPN-79". In  
one aspect, the invention provides a substantially purified polypeptide comprising an  
amino acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2,  
10 SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID  
NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13,  
SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ  
ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID  
NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29,  
15 SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ  
ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID  
NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45,  
SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ  
ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID  
NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61,  
20 SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ  
ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID  
NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77,  
SEQ ID NO:78, and SEQ ID NO:79 (SEQ ID NO:1-79), and fragments thereof.  
25 The invention further provides a substantially purified variant having at least 90%  
amino acid identity to at least one of the amino acid sequences selected from the group  
consisting of SEQ ID NO:1-79, and fragments thereof. The invention also provides an  
30

isolated and purified polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-79, and fragments thereof. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-79, and fragments thereof.

Additionally, the invention provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-79, and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:1-79, and fragments thereof.

The invention also provides an isolated and purified polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:112, SEQ ID NO:113, SEQ ID NO:114, SEQ ID NO:115, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, SEQ ID NO:126, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:142, SEQ ID NO:143, SEQ ID NO:144, SEQ ID NO:145, SEQ ID NO:146, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:150, SEQ ID NO:151, SEQ ID NO:152, SEQ ID NO:153, SEQ ID NO:154, SEQ ID NO:155, SEQ ID NO:156, SEQ ID NO:157, and SEQ ID NO:158 (SEQ ID NO:80-158), and fragments thereof. The invention further provides an isolated and purified polynucleotide variant having at least

90% polynucleotide sequence identity to the polynucleotide sequence selected from the group consisting of SEQ ID NO:80-158, and fragments thereof. The invention also provides an isolated and purified polynucleotide having a sequence which is complementary to the polynucleotide comprising a polynucleotide sequence selected from 5 the group consisting of SEQ ID NO:80-158, and fragments thereof.

The invention also provides a method for detecting a polynucleotide in a sample containing nucleic acids, the method comprising the steps of (a) hybridizing the complement of the polynucleotide sequence to at least one of the polynucleotides of the sample, thereby forming a hybridization complex; and (b) detecting the hybridization 10 complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide in the sample. In one aspect, the method further comprises amplifying the polynucleotide prior to hybridization.

The invention further provides an expression vector containing at least a fragment 15 of the polynucleotide encoding the polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-79, and fragments thereof. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide, the method comprising the steps of: (a) culturing the host cell containing an expression vector containing at least a fragment of a polynucleotide under conditions suitable for the 20 expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the amino acid sequence selected from the group 25 consisting of SEQ ID NO:1-79, and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide selected from the group consisting of SEQ ID NO:1-79, and fragments thereof. The invention also provides a purified agonist and a purified antagonist to the polypeptide.

The invention also provides a method for treating or preventing a disorder 30 associated with decreased expression or activity of HTMPN, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising a substantially purified polypeptide having the

amino acid sequence selected from the group consisting of SEQ ID NO:1-79, and fragments thereof, in conjunction with a suitable pharmaceutical carrier.

The invention also provides a method for treating or preventing a disorder associated with increased expression or activity of HTMPN, the method comprising  
5 administering to a subject in need of such treatment an effective amount of an antagonist of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-79, and fragments thereof.

#### BRIEF DESCRIPTION OF THE TABLES

10 Table 1 shows nucleotide and polypeptide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone ID), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding HTMPN.

Table 2 shows features of each polypeptide sequence including predicted transmembrane sequences, potential motifs, homologous sequences, and methods and  
15 algorithms used for identification of HTMPN.

Table 3 shows the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis, diseases, disorders, or conditions associated with these tissues, and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which  
20 Incyte cDNA clones encoding HTMPN were isolated.

Table 5 shows the programs, their descriptions, references, and threshold parameters used to analyze HTMPN.

#### DESCRIPTION OF THE INVENTION

25 Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the  
30 appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

#### DEFINITIONS

"HTMPN" refers to the amino acid sequences of substantially purified HTMPN obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which, when bound to HTMPN, increases or prolongs the duration of the effect of HTMPN. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of HTMPN.

An "allelic variant" is an alternative form of the gene encoding HTMPN. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding HTMPN include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same as HTMPN or a polypeptide with at least one functional characteristic of

HTMPN. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding HTMPN, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding HTMPN.

- 5 The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent HTMPN. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of
- 10 HTMPN is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

- 15 The terms "amino acid" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of HTMPN which are preferably at least 5 to about 15 amino acids in length, most preferably at least 14 amino acids, and which retain
- 20 some biological activity or immunological activity of HTMPN. Where "amino acid sequence" is recited to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

- 25 "Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

- 30 The term "antagonist" refers to a molecule which, when bound to HTMPN, decreases the amount or the duration of the effect of the biological or immunological activity of HTMPN. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of HTMPN.

The term "antibody" refers to intact molecules as well as to fragments thereof, such

as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind HTMPN polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) 5 can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that fragment of a molecule (i.e., an 10 epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for 15 binding to an antibody.

The term "antisense" refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once introduced into a cell, the complementary nucleotides combine with natural 20 sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

The term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically 25 active" refers to the capability of the natural, recombinant, or synthetic HTMPN, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity" refer to the natural binding of 30 polynucleotides by base pairing. For example, the sequence "5' A-G-T 3'" bonds to the complementary sequence "3' T-C-A 5'." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules.

The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

- 5 A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding HTMPN or fragments of HTMPN may be employed as hybridization probes.
- 10 The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).
- "Consensus sequence" refers to a nucleic acid sequence which has been  
15 resequenced to resolve uncalled bases, extended using XL-PCR kit (Perkin-Elmer, Norwalk CT) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW Fragment Assembly system (GCG, Madison WI). Some sequences have been both extended and assembled to  
20 produce the consensus sequence.

The term "correlates with expression of a polynucleotide" indicates that the detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding HTMPN, by northern analysis is indicative of the presence of nucleic acids encoding HTMPN in a sample, and thereby correlates with expression of the transcript  
25 from the polynucleotide encoding HTMPN.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide  
30 sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is

one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

- The term "similarity" refers to a degree of complementarity. There may be partial similarity or complete similarity. The word "identity" may substitute for the word "similarity." A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as "substantially similar." The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction.
- 15 The absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases "percent identity" or "% identity" refer to the percentage of sequence similarity found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MEGALIGN program (DNASTAR, Madison WI) which creates alignments between two or more sequences according to methods selected by the user, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known

in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

“Human artificial chromosomes” (HACs) are linear microchromosomes which 5 may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance.

The term “humanized antibody” refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

10 “Hybridization” refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

The term “hybridization complex” refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C<sub>0</sub>t or R<sub>0</sub>t analysis) or 15 formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

20 The words “insertion” or “addition” refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

“Immune response” can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other 25 signaling molecules, which may affect cellular and systemic defense systems.

The term “microarray” refers to an arrangement of distinct polynucleotides on a substrate.

The terms “element” or “array element” in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

30 The term “modulate” refers to a change in the activity of HTMPN. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of HTMPN.

The phrases "nucleic acid" or "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to 5 any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

The terms "operably associated" or "operably linked" refer to functionally related 10 nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator 15 sequences that control expression of the polypeptide.

The term "oligonucleotide" refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. "Oligonucleotide" is substantially equivalent to the terms 20 "amplimer," "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers 25 solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

The term "sample" is used in its broadest sense. A sample suspected of containing 30 nucleic acids encoding HTMPN, or fragments thereof, or HTMPN itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" or "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody 5 is specific for epitope "A," the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "stringent conditions" refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be 10 defined by salt concentration, the concentration of organic solvent, e.g., formamide, temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

The term "substantially purified" refers to nucleic acid or amino acid sequences 15 that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acids or 20 nucleotides by different amino acids or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or 25 polypeptides are bound.

"Transformation" describes a process by which exogenous DNA enters and 30 changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes 35 stably transformed cells in which the inserted DNA is capable of replication either as an

autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of HTMPN polypeptides refers to an amino acid sequence that is altered by one or more amino acid residues. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR).

The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to HTMPN. This definition may also include, for example, "allelic" (as defined above), "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

## THE INVENTION

The invention is based on the discovery of new human transmembrane proteins (HTMPN), the polynucleotides encoding HTMPN, and the use of these compositions for the diagnosis, treatment, or prevention of immune, reproductive, smooth muscle, neurological, gastrointestinal, developmental, and cell proliferative disorders.

Table 1 lists the Incyte Clones used to derive full length nucleotide sequences encoding HTMPN. Columns 1 and 2 show the sequence identification numbers (SEQ ID

NOs) of the amino acid and nucleic acid sequences, respectively. Column 3 shows the Clone ID of the Incyte Clone in which nucleic acids encoding each HTMPN were identified, and column 4, the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones, their corresponding cDNA libraries, and shotgun sequences. The clones and shotgun sequences are part of the consensus nucleotide sequence of each HTMPN and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3, potential phosphorylation sites; column 4, 10 potential glycosylation sites; column 5, the amino acid residues comprising signature sequences and motifs; column 6, the identity of each protein; and column 7, analytical methods used to identify each protein through sequence homology and protein motifs. Hidden Markov Model analysis indicates the presence of one or more potential transmembrane motifs in each of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO: 66, SEQ 15 ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO: 75, SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO: 79; as well as the presence of one or more potential signal peptide motifs in each of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:77, and SEQ ID 20 NO:79.

Motifs analysis indicates the presence of a potential ATP/GTP binding site in SEQ ID NO:68, a potential calcium-binding site also in SEQ ID NO:68, a potential leucine zipper gene regulatory motif in each of SEQ ID NO:68 and SEQ ID NO:73; and a potential microbody (single-membraned organelle) targeting signal site in SEQ ID NO:78. 25 BLOCKS analysis indicates the presence of two potential PMP-22 integral membrane glycoprotein motifs and a trehalase motif, all in SEQ ID NO:77, as well as a potential protein-splicing motif in SEQ ID NO:66. PRINTS analysis indicates the presence of a potential G-protein coupled receptor motif in SEQ ID NO:79.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or 30 conditions associated with nucleotide sequences encoding HTMPN. The first column of Table 3 lists the nucleotide sequence identifiers. The second column lists tissue categories which express HTMPN as a fraction of total tissue categories expressing HTMPN. The

third column lists the diseases, disorders, or conditions associated with those tissues expressing HTMPN. The fourth column lists the vectors used to subclone the cDNA library. Of particular note is the expression of HTMPN in tissue involved in inflammation and the immune response and with cell proliferative conditions including cancer, and in 5 reproductive, gastrointestinal, fetal, smooth muscle, cardiovascular, urologic, endocrine, developmental, and nervous tissue.

The following fragments of the nucleotide sequences encoding HTMPN are useful in hybridization or amplification technologies to identify SEQ ID NO:121-158 and to distinguish between SEQ ID NO:121-158 and related polynucleotide sequences. The 10 useful fragments are the fragment of SEQ ID NO:121 from about nucleotide 151 to about nucleotide 189; the fragment of SEQ ID NO:122 from about nucleotide 280 to about nucleotide 318; the fragment of SEQ ID NO:123 from about nucleotide 505 to about nucleotide 558; the fragments of SEQ ID NO:124 from about nucleotide 1 to about nucleotide 21 and from about nucleotide 694 to about nucleotide 720; the fragment of SEQ 15 ID NO:125 from about nucleotide 331 to about nucleotide 378; the fragment of SEQ ID NO:126 from about nucleotide 1012 to about nucleotide 1047; the fragment of SEQ ID NO:127 from about nucleotide 1070 to about nucleotide 1106; the fragment of SEQ ID NO:128 from about nucleotide 133 to about nucleotide 186; the fragment of SEQ ID NO:129 from about nucleotide 432 to about nucleotide 482; the fragments of SEQ ID 20 NO:130 from about nucleotide 1745 to about nucleotide 1795 and from about nucleotide 1910 to about nucleotide 1979; the fragment of SEQ ID NO:131 from about nucleotide 322 to about nucleotide 375; the fragment of SEQ ID NO:132 from about nucleotide 147 to about nucleotide 203; the fragment of SEQ ID NO:133 from about nucleotide 557 to about nucleotide 613; the fragment of SEQ ID NO:134 from about nucleotide 509 to about 25 nucleotide 595; the fragment of SEQ ID NO:135 from about nucleotide 808 to about nucleotide 848; the fragment of SEQ ID NO:136 from about nucleotide 216 to about nucleotide 260; the fragment of SEQ ID NO:137 from about nucleotide 132 to about nucleotide 188; the fragment of SEQ ID NO:138 from about nucleotide 231 to about nucleotide 278; the fragment of SEQ ID NO:139 from about nucleotide 303 to about 30 nucleotide 350; the fragment of SEQ ID NO:140 from about nucleotide 507 to about nucleotide 550; the fragment of SEQ ID NO:141 from about nucleotide 433 to about nucleotide 477; the fragment of SEQ ID NO:142 from about nucleotide 266 to about

nucleotide 314; the fragment of SEQ ID:143 from about nucleotide 3 to about nucleotide 48; the fragment of SEQ ID NO:144 from about nucleotide 76 to about nucleotide 122; the fragment of SEQ ID NO:145 from about nucleotide 93 to about nucleotide 139; the fragment of SEQ ID NO:146 from about nucleotide 241 to about nucleotide 286; the 5 fragment of SEQ ID NO:147 from about nucleotide 43 to about nucleotide 89; the fragment of SEQ ID NO:148 from about nucleotide 219 to about nucleotide 265; the fragment of SEQ ID NO:149 from about nucleotide 619 to about nucleotide 663; the fragment of SEQ ID NO:150 from about nucleotide 25 to about nucleotide 69; the fragment of SEQ ID NO:151 from about nucleotide 175 to about nucleotide 221; the 10 fragment of SEQ ID NO:152 from about nucleotide 94 to about nucleotide 138; the fragment of SEQ ID NO:153 from about nucleotide 46 to about nucleotide 90; the fragment of SEQ ID NO:154 from about nucleotide 1081 to about nucleotide 1127; the fragment of SEQ ID NO:155 from about nucleotide 31 to about nucleotide 77; the fragment of SEQ ID NO:156 from about nucleotide 157 to about nucleotide 201; the 15 fragment of SEQ ID NO:157 from about nucleotide 216 to about nucleotide 259; and the fragment of SEQ ID NO:158 from about nucleotide 517 to about nucleotide 561. The polypeptides encoded by these fragments may be useful, for example, as antigenic polypeptides.

The invention also encompasses HTMPN variants. A preferred HTMPN variant is 20 one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the HTMPN amino acid sequence, and which contains at least one functional or structural characteristic of HTMPN.

The invention also encompasses polynucleotides which encode HTMPN. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising 25 a sequence selected from the group consisting of SEQ ID NO:80-158, which encodes HTMPN.

The invention also encompasses a variant of a polynucleotide sequence encoding HTMPN. In particular, such a variant polynucleotide sequence will have at least about 80%, more preferably at least about 90%, and most preferably at least about 95% 30 polynucleotide sequence identity to the polynucleotide sequence encoding HTMPN. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:80-158 which

has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:80-158. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or 5 structural characteristic of HTMPN.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding HTMPN, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every 10 possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring HTMPN, and all such variations are to be considered as being specifically disclosed.

15 Although nucleotide sequences which encode HTMPN and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring HTMPN under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding HTMPN or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons.  
20 Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HTMPN and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable 25 properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode HTMPN and HTMPN derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available 30 expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding HTMPN or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:80-158 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. 5 (1987) Methods Enzymol. 152:507-511.) For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency 10 hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and 15 the inclusion or exclusion of carrier DNA, are well known to those skilled in the art.

Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 20 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash 25 stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the 30 wash steps will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In

a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

- 5 Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Perkin-Elmer), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading
- 10 exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the Hamilton MICROLAB 2200 (Hamilton, Reno NV), Peltier Thermal Cycler 200 (PTC200; MJ Research, Watertown MA) and the ABI CATALYST 800 (Perkin-Elmer). Sequencing is then carried out using either ABI 373 or 377 DNA
- 15 sequencing systems (Perkin-Elmer) or the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA). The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY,
- 20 pp. 856-853.)

The nucleic acid sequences encoding HTMPN may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to

25 amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this

method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306).  
5 Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06  
10 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in  
15 which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic  
20 separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Perkin-Elmer), and the entire process from loading of samples to computer analysis and electronic data display may be computer  
25 controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode HTMPN may be cloned in recombinant DNA molecules that direct expression of HTMPN, or fragments or functional equivalents thereof, in appropriate host  
30 cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express HTMPN.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter HTMPN-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding HTMPN may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Res. Symp. Ser. 215-223, and Horn, T. et al. (1980) Nucl. Acids Res. Symp. Ser. 225-232.) Alternatively, HTMPN itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Additionally, the amino acid sequence of HTMPN, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY.)

In order to express a biologically active HTMPN, the nucleotide sequences encoding HTMPN or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding HTMPN. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding HTMPN. Such signals include the ATG initiation codon and adjacent sequences, e.g. the

Kozak sequence. In cases where sequences encoding HTMPN and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous 5 translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

10 Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding HTMPN and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, 15 Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express 20 sequences encoding HTMPN. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

25 In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding HTMPN. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding HTMPN can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or pSPORT1 plasmid (Life Technologies).

30 Ligation of sequences encoding HTMPN into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be

useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of HTMPN are needed, e.g. for the production of antibodies, vectors which direct high level expression of HTMPN may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of HTMPN. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris.

- 10 In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Grant et al. (1987) Methods Enzymol. 153:516-54; and Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of HTMPN. Transcription of sequences encoding HTMPN may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized.

- 25 In cases where an adenovirus is used as an expression vector, sequences encoding HTMPN may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses HTMPN in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., 5 Harrington, J.J. et al. (1997) *Nat Genet.* 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of HTMPN in cell lines is preferred. For example, sequences encoding HTMPN can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker 10 gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated 15 using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk* or *apr* cells, respectively. (See, e.g., Wigler, M. et al. (1977) *Cell* 11:223-232; Lowy, I. et al. (1980) *Cell* 22:817-823.) 20 Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides, neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) *Proc. Natl. Acad. Sci.* 77:3567-3570; Colbere-Garapin, F. et al. (1981) *J. Mol. Biol.* 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, 25 which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) *Proc. Natl. Acad. Sci.* 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These 30 markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) *Methods Mol. Biol.* 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding HTMPN is inserted within a marker gene sequence, transformed cells containing sequences encoding HTMPN can be identified by the absence 5 of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding HTMPN under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding HTMPN and 10 that express HTMPN may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

15        Immunological methods for detecting and measuring the expression of HTMPN using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two 20 non-interfering epitopes on HTMPN is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul MN, Sect. IV; Coligan, J. E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, 25 Humana Press, Totowa NJ).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding HTMPN include oligolabeling, nick translation, end-labeling, or 30 PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding HTMPN, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be

used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or 5 labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding HTMPN may be cultured under conditions suitable for the expression and recovery of the protein from cell 10 culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode HTMPN may be designed to contain signal sequences which direct secretion of HTMPN through a prokaryotic or eukaryotic cell membrane.

15 In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "pro" form of the protein may also be used to specify protein targeting, 20 folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC, Bethesda MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

25 In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding HTMPN may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric HTMPN protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for 30 inhibitors of HTMPN activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose

binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and 5 hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the HTMPN encoding sequence and the heterologous protein sequence, so that HTMPN may be cleaved away from the heterologous moiety 10 following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, *supra*, ch 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled HTMPN may be achieved *in vitro* using the TNT rabbit reticulocyte lysate or wheat germ extract 15 systems (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably  $^{35}\text{S}$ -methionine.

Fragments of HTMPN may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, *supra*, 20 pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin-Elmer). Various fragments of HTMPN may be synthesized separately and then combined to produce the full length molecule.

## THERAPEUTICS

25 Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of HTMPN and human transmembrane proteins. In addition, the expression of HTMPN is closely associated with tissue involved in inflammation and the immune response and with cell proliferative conditions including cancer, and in reproductive, gastrointestinal, fetal, smooth muscle, cardiovascular, developmental, and 30 nervous tissue. Therefore, HTMPN appears to play a role in immune, reproductive, smooth muscle, neurological, gastrointestinal, developmental, and cell proliferative disorders. In the treatment of immune, reproductive, smooth muscle, neurological,

gastrointestinal, developmental, and cell proliferative disorders associated with increased HTMPN expression or activity, it is desirable to decrease the expression or activity of HTMPN. In the treatment of the above conditions associated with decreased HTMPN expression or activity, it is desirable to increase the expression or activity of HTMPN.

- 5 Therefore, in one embodiment, HTMPN or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of HTMPN. Examples of such disorders include, but are not limited to, an immune disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, 10 anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, 15 glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, 20 thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a reproductive disorder such as a disorder of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; a disruption of the estrous cycle, a disruption of the menstrual cycle, 25 polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the 30 male breast, and gynecomastia; a smooth muscle disorder such as angina, anaphylactic shock, arrhythmias, asthma, cardiovascular shock, Cushing's syndrome, hypertension, hypoglycemia, myocardial infarction, migraine, and pheochromocytoma, and myopathies

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